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INSPIREd to take up dental research

by Helen Petersen and Rupert Austin

Winning abstracts from the INSPIRE conference.



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On 24 October 2015, the first INSPIRE conference for dental students was held at Guy's Hospital London. The INSPIRE scheme was set up by the Academy of Medical Sciences and the Wellcome Trust to encourage medical and dental students to think about a career in clinical academia. On behalf dentistry, a grant was won by Dr Helen Petersen and Dr Rupert Austin as part of the INSPIRE special project scheme to host the conference.

The meeting was a huge success and was attended by over 60 students from up and down the country. The attendees, or 'Research Champions,' were students who had been specially selected by their dental schools. They enjoyed a day of talks from leading academics in dentistry and had a chance to present their own research, with some selected for oral presentation and the rest presented as posters. The Research Champions heard presentations on how to get started on a clinical academic career and how to be a success, plus tips on networking, using social media and mentoring.

Finally, the top six student abstracts were selected for oral presentation and judged by the senior academics attending the event. The standard was exceptionally high, and the winners were Catherine Liu from King's College London Dental Institute, Hannah Brownrigg-Gleeson from Cardiff University, School of Dentistry, and Paul Hankinson from Sheffield University.

The next stage of the INSPIRE award sees the Research Champions organising their own local conferences within their dental schools, to inspire the rest of their student colleagues about the fantastic career clinical academia can offer.

Validation of a low-cost portable device for 3D facial imaging
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Introduction: Three-dimensional (3D) facial imaging is useful in many clinical applications to record soft tissue surface data. A widely used technique for routine facial imaging is 3D digital stereophotogrammetry. Scanify (Fuel 3D Technologies, Ltd, Chinnor, UK) is a newly released

ultra-low-cost optical scanning device combined with photometric stereo imaging. The aim of this study was to validate the device for the 3D imaging of facial casts.

Materials and Methods: Two facial casts, obtained by recording impressions of two subjects, were marked with anthropometric landmarks then digitised repeatedly using Scanify and a previously validated portable digital stereophotogrammetry device (Vectra H1; Canfield Scientific, Inc., Fairfield, NJ, USA). The 3D images acquired with each device were compared using linear interlandmark measurements and 3D surface analysis software.

Results: Comparing the two sets of images, we found that 91% of interlandmark measurements were within 1 mm both of each other and of the corresponding reference values acquired by digital calipers. Mean overall surface difference was <0.3 mm. Significant differences were found in depth measurements, as illustrated by colour maps of surface deviations. Scanify data produced significantly greater registration errors when merging multiple images (1 mm) versus Vectra H1 (0.3 mm) ($p < 0.01$).

Conclusion: Scanify images of facial casts were comparable to Vectra H1 in accuracy and reliability on a millimetre scale when imaging simple topography, but showed significant errors on depth measurements. Merging multiple images increased the field of view but also the imaging error. While the Scanify device could therefore be considered for some clinical applications, further development is required.

Keywords: Anthropometry, 3D imaging, Face, Photogrammetry

The effect of contemporary dental restorative materials on dental pulp progenitor cells

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Introduction: The dentine-pulp complex demonstrates compelling regenerative potential following injury or trauma that is attributable to residing bioactive molecules bound within the dentine extracellular matrix.

Studies have revealed multipotent mesenchymal stem cells, known as dental pulp stem cells (DPSCs), that are recognised as essential to dentinogenesis following injury. The synergistic interactions of endogenous bioactive molecules, including growth factors, are known to be released during injury, inducing proliferation and differentiation of DPSCs into a new generation of dentine-secreting cells, odontoblasts. Clinical practice now uses restorative materials to encourage the regenerative nature of dentine. However, there is a lack of biological evidence to support such clinical application, potentially exposing patients to risks. This study aimed to investigate and quantify the capability of routinely used clinical conditioning agents to augment the release and exposure of dentine matrix proteins (DMPs) from cut dentine surfaces. The subsequent ability of DMPs to induce and promote the differentiation of DPSCs seeded onto dentine surfaces treated with calcium hydroxide were explored.

Materials and Methods: 1–2 mm dentine sections were cut from human molar teeth and subsequently chemically treated with 10% ethylenediaminetetraacetate (EDTA), 10% citric acid, 10% phosphoric acid, 0.02M calcium hydroxide or phosphate-buffered saline (PBS) for 5 and 10 minutes. Following treatment, enzyme-linked immunosorbent assay was used to quantify the concentration of transforming growth factor beta (TGF- β), vascular endothelial growth factor (VEGF) and Bone morphogenetic protein (BMP)-2 released, while immunogold labelling and scanning electron microscope (SEM) images measured exposure of VEGF and BMP-2. The seeding of DPSCs onto calcium hydroxide-treated dentine were observed after 1, 4 and 14 days under light microscopy.

Results: The release and presence of growth factors was observed with all chemical treatments compared to PBS and negative controls. EDTA was effective at releasing TGF- β , while citric acid was the most effective at releasing and exposing VEGF and BMP-2. SEM revealed extensive etching with all acidic treatments. All treatments with acids showed a decrease in growth factor exposure following treatment for 10 minutes. Calcium hydroxide demonstrated successful exposure and release. Phosphoric acid was the conditioning agent least able to induce growth factor release and exposure. Cells seeding onto treated dentine firmly attached to the dentine surface, while taking on an odontoblast-like morphology.

Conclusion: Citric acid proved the most effective treatment for the release and exposure of DMPs after 5 minutes of treatment, which is considered more clinically feasibility. EDTA was associated with sufficient DMP release, while Calcium hydroxide increased osteogenic potential on dentine surfaces. Phosphoric acid exhibited little ability to release DPSCs. This data may provide a basis of selecting innovative and novel clinical therapies for the optimal exploitation of the regenerative capacity of the dentine-pulp complex.

Keywords: Cell Differentiation, Dental Pulp, Dentin, Dentinogenesis, Extracellular Matrix, Odontoblasts

The role of CALML3 in oral squamous cell carcinoma

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Introduction: The aims of the project were to determine the expression of calmodulin-like protein 3 (CALML3) mRNA and protein in a cohort of normal oral keratinocytes (NOK), immortalised normal oral keratinocytes (iNOK), oral potentially malignant lesion (OPML) and oral squamous cell carcinoma cell (OSCC) lines. Moreover, to measure ex vivo expression, in normal oral mucosa, OPML and OSCC by immunohistochemistry (IHC). Furthermore, we wanted to investigate CALML3's role in immortalisation and differentiation. We also aimed to determine the phenotypic changes resulting from overexpression of CALML3 in OPML and OSCC cell lines where expression had been lost.

Materials and Methods: The mRNA and protein expression of CALML3 were measured by real-time polymerase chain reaction (qPCR) and western blot in NOK, iNOK, OPML and OSCC derived cell lines. Ex vivo expression was measured in normal oral mucosa, OPML and OSCC by IHC. The relationship of CALML3 expression to that of involucrin (a marker of differentiation) and myosin-X (a protein related to CALML3 activity) was also determined by qPCR and western blot respectively. The effects of re-expressing CALML3 in OPML and OSCC cell lines were investigated using transient transfection using the pCMV6-CALML3 vector. The effects of expression of CALML3 on cell migration and proliferation were assessed via cell exclusion assay and an 5-ethynyl-2'-deoxyuridine-based proliferation assay, respectively. Also, changes in involucrin and myosin-X expression following transfection were determined.

Results: Down regulation of CALML3 was found in OSCC and OPML, as well as similar localisation in tissues, to that seen previous studies. Its expression was the same in both OPML and OSCC. Its staining was highly variable in OSCC, with, for example, increased expression in superficial keratinocytes of the oral epithelium. CALML3 expression varied greatly in immortalised cell lines. It was also found that expression of CALML3 correlated with the expression of involucrin. Furthermore, CALML3 re-expression had no effect on the migration of OSCC cells. Finally, re-expression of CALML3 had no effects on the proliferation or expression of involucrin.

Conclusions: This study has shown CALML3 to be down regulated in OPML and OSCC and myosin-x expression is increased in some OPML and OSCC cell lines. Moreover, CALML3 expression is unlikely to be involved in immortalisation but rather differentiation. It is not important in migration of the OSCC cell line tested and this may be due to high myosin-x expression in this cell line identified in a previous study. It is unlikely that CALML3 would have use as a biomarker of malignancy. The reason for its down regulation in cancers remains enigmatic and may be due to its links with differentiation.

Keywords: Calmodulin, Cell Differentiation, Keratinocytes, Squamous cell carcinoma